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Neuregulins as Potential Neuroprotective Agents

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INTRODUCTION

Neuron-glia interactions are important for many aspects of nervous system development, maintenance and function. Generation of the appropriate types and numbers of neural cells is regulated by cell-cell signaling events involving soluble and cell-attached factors, which promote cell survival and proliferation. Patterning and morphogenesis in the embryo, and the ability of cells to progress along specific lineages, also are regulated by local interactions and environmental influences. Expression of terminal differentiated phenotypes also are subject to various forms of inductive signaling. Peptide growth factors and receptor tyrosine kinases are central to many of these processes, and in the nervous system the neuregulins and their receptors are an important part of the story (see Refs. 1, 2 for reviews).

The neuregulins, also known as glial growth factor (GGF), acetylcholine receptor-inducing activity (ARIA), neu differentiation factor (NDF), and heregulins (HRGs), are all products of a single neuregulin gene.[3-6] The name neuregulins, which refers to the entire group of these gene products, reflects signaling through *neu* and related receptors and points to a significant involvement of these molecules in regulating neural development and function. The neuregulins belong to the epidermal growth factor (EGF) superfamily and exhibit a great variety of membrane-attached and secreted isoforms generated by alternative splicing and usage of three distinct promoters (FIG. 1). Neuregulins mediate their biological activities through members of the EGF receptor family, erbB2, erbB3 and erbB4. Disruptions of the neuregulin erbB2 and erbB4 genes have shed additional light on the molecular

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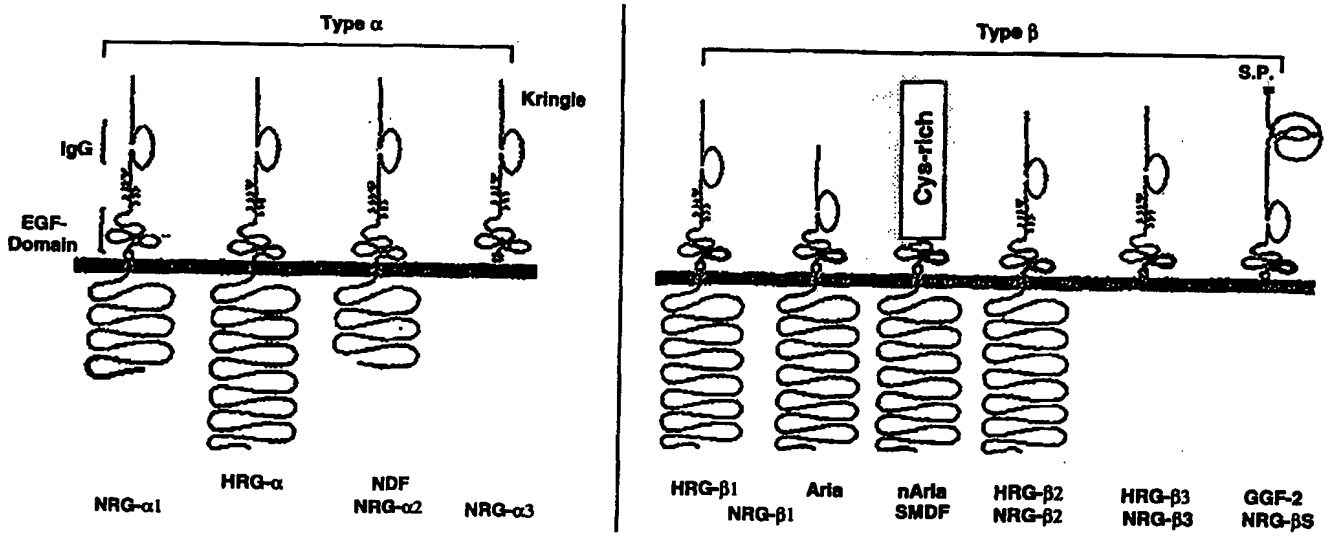


FIGURE 1. A schematic representation of the neuregulins. Common structural elements include an immunoglobulin fold and one EGF-like domain. The diversity of forms is generated by several molecular mechanisms: 1) alternate promoter usage leads to at least three mutually exclusive N-termini—one containing a signal peptide and a potential kringle domain, a second with a hydrophilic sequence and a third that has eight closely-spaced cysteine residues;[30] 2) at least three distinct C-termini are used, which are derived from unique stop codons, 3′ untranslated sequences and polyadenylation sites; 3) alternative splicing generates forms that either contain or lack a linker sequence rich in serines and threonines that separate the immunoglobulin (Ig) domain and the EGF-like domain, and produces two alternative EGF-like folds (α and β), which differ in the third cysteine loop. Several alternative cleavage sites adjacent to the transmembrane segment also are encoded by different DNA sequences. (Adapted from Peles & Yarden.[31])

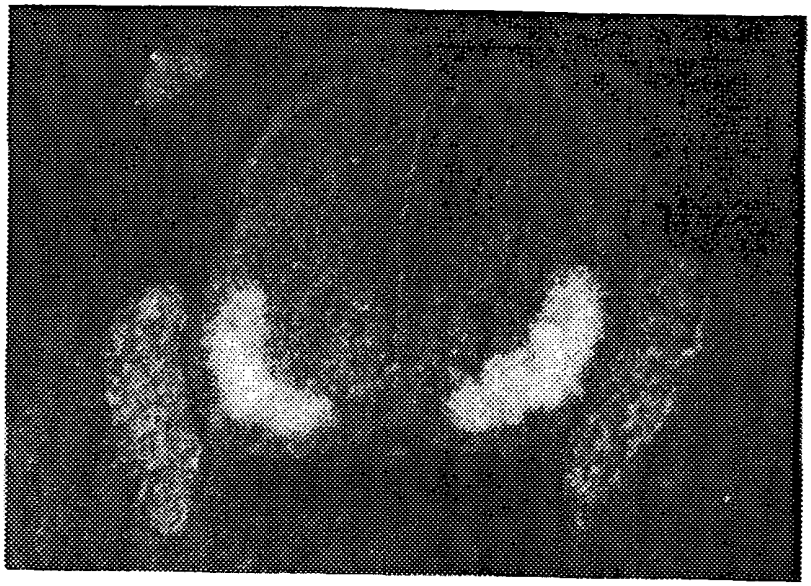


FIGURE 2. Expression of neuregulin transcripts in the developing spinal cord. A section of spinal cord taken from a day 13 mouse embryo was hybridized with a rat antisense probe as described.[5]

interactions of this signaling system, and indicate that all three genes are essential for development of both the heart and the hind brain.[7-9]

The diverse isoforms may support multiple modes of delivering these peptide growth factors to target cells, such as paracrine, juxtacrine, endocrine and autocrine modes of signaling. In the nervous system, neuregulin isoforms are expressed by both peripheral and central neurons and are involved in numerous interactions between neurons, glia and other target tissues. Expression sites in the nervous system have been mapped by *in situ* hybridization,[10,11] and neuregulin immunoreactivity has been localized in the brain,[12] at motor endplates,[13] in the spinal cord and along peripheral axons,[14] and in the retina.[15] There is particularly strong expression in motor neurons in the ventral horn of the spinal cord, and in sensory neurons in dorsal root ganglia (FIG. 2).

Neuregulins are turning out to be important mediators of cell communication in the nervous system. We have used both *in vitro* and *in vivo* approaches to characterize specific neural activities of a secreted form of neuregulin, recombinant human glial growth factor 2 (rhGGF2), and summarize here our results on two important roles of neuregulins in neuron-glial interactions. The first relates to regulation of the number of glial cells; the second deals with the provision of trophic support to neurons from glia. Neuregulin signaling promotes survival and proliferation of peripheral and central glial cells and their progenitors,[5,12,16-25] and restricts multipotential neural crest stem cells to a glial cell fate.[26] One or more of these biological activities presumably accounts for the absence of erbB3-expressing Schwann cells along outgrowing peripheral axons in neuregulin-deficient mice.[7] Interestingly, neuregulins stimulate the expression of soluble neurotrophic activities

in nonneuronal cells of embryonic sympathetic ganglia,[27] and in Schwann cells cultured from neonatal sciatic nerve.[28] By participating in a trophic support loop between neurons and glia, neuregulins may have indirect neurotrophic effects. These findings indicate that neuregulin/rhGGF2 may have clinical utility in the treatment of certain neurological disorders.

RESULTS

Neuregulins Regulate the Number of Central and Peripheral Glial Cells

Control of cell number is critically important for numerous aspects of embryonic development including that of glial cells (see Ref. 31). In mature axon-glial interactions, there exists an optimal ratio of myelin forming cells to axons, which is required to maintain saltatory impulse conduction and other essential functions. Several mechanisms regulate the size of any population of differentiated cells, including specification to a selected lineage from a multipotential progenitor, migration, proliferation, death, and dedifferentiation. We have investigated the role of neuregulins in regulating glial cell number in both central and peripheral systems, and find that neuronally produced neuregulins are involved in all of these processes.

Neuregulins Regulate Oligodendroglial Development

Oligodendrocyte progenitors arise from the subventricular zone (SVZ) and migrate out from there into developing white matter, differentiating and proliferating en route.[29,30] From the time these cells migrate out from the SVZ and come to reside in white matter tracts where they myelinate axons, they are directly in contact with neurons or their axons. Further, a number of studies indicate that neurons profoundly influence the survival, proliferation and differentiation of cells of the oligodendrocyte lineage (reviewed in Refs. 31, 32). Since neuregulins are expressed in the SVZ[33] and in many regions of the brain,[10–12] these growth factors may signal some of these key events in oligodendroglial development. Thus, we have investigated the effects of rhGGF2 on the proliferation, survival and differentiation of oligodendrocyte progenitors, which were established in culture from neonatal rat cortex as described previously.[34]

A highly purified population of O-2A progenitor cells, depleted of neurons and type 1 astrocytes, was expanded in media supplemented with B104 conditioned media (CM). These cells had a bipolar morphology and remained highly proliferative, and >95% of the cells expressed markers characteristic of O-2A progenitors, such as vimentin and A2B5, but not markers of more mature cells in the glial lineage, such as O4, O1 or glial fibrillary acidic protein (GFAP). When switched to a defined medium plus 0.5% serum (DM^+), the cells rapidly acquired a multipolar morphology and began to express the O4 antigen as an early sign of differentiation. After several days, the cells developed a complex network of processes and began to express the O1 antigen, which is characteristic of mature oligodendrocytes. By 3 days in DM^+, essentially all cells were O4+ and over 50% were O1+. This process of differentiation coincided with reduced proliferation: the initial labeling index of O-2A progenitor cells grown in B104 conditioned media was greater than 50% after a 4 hour pulse of BrdU, but decreased to less than 5% when cells were grown in DM^+ for 3 days.

Neuregulin is a Potent Mitogen for Cells of the Oligodendrocyte Lineage

To characterize the effects of rhGGF2, cells grown in DM$^+$ for 3 days were treated with 200 ng/ml of rhGGF2 for 16 hours, the last four hours in the presence of bromodeoxyuridine (BrdU). Cells were then fixed and stained for O4, O1 and BrdU. The results (FIG. 3) demonstrate that all cells remained O4+ (panel B) but most rhGGF2-treated cells had a less complex morphology than control cells (panel A), with fewer and flatter processes suggestive of a less mature phenotype. Also, significantly fewer O1+ cells were present after rhGGF2 treatment compared to controls (panels C and D). There was a substantial increase in the number of BrdU-positive nuclei in the treated compared to the control cells (panels E and F). The labeling index was determined separately for O4+ and O1+ cells (FIG. 3G), which shows that rhGGF2 was a potent mitogen for O4+ cells, but less so for more mature (01-positive) cells (FIG. 3H).

Neuregulin Promotes Oligodendrocyte Progenitor Survival

Survival of oligodendrocyte progenitors *in vitro* is dependent on the presence of a number of growth factors or low concentrations of serum (*e.g.*, Ref. 35). To examine directly whether rhGGF2 had survival effects for oligodendrocyte progenitors, cells were grown in DM$^+$ for three days, then switched to a serum-free defined media containing varying concentrations of rhGGF2 for an additional 12 or 24 hours to analyze cell death (FIG. 4). In the absence of rhGGF2 approximately 50% of the cells were dead at 12 hours. By contrast, at 200 ng/ml of rhGGF2 only about 10% of the cells were dead. The survival effect of rhGGF2 on O4+ cells was dose dependent with a half maximal response of approximately 2 ng/ml.

Further studies[23,36] have shown that high levels of rhGGF2 reversibly inhibit the differentiation of oligodendrocyte progenitors beyond the O4+/O1− stage, inhibit their lineage commitment and promote a reversion of O1+ cells to the O4+/O1− stage associated with a striking increase in the expression of nestin, a marker of less mature cells in this lineage. Neuregulin receptors are expressed by cells at different stages in the lineage and are activated by rhGGF2. Interestingly, there is a transition in receptor isoforms as O-2A cells differentiate into oligodendrocytes. Finally, cortical neurons release a soluble mitogen for prooligodendrocytes that is specifically blocked by antibodies to rhGGF2. These results implicate neuregulins in the neuronal regulation of oligodendrocyte progenitor proliferation, survival and differentiation, thus supporting the role of neuregulins in regulating glial cell number in the central nervous system (CNS).

Neuregulins Regulate the Number of Schwann Cells in Peripheral Nerve

The GGFs were discovered as a result of an effort to identify sources of Schwann cell mitogenic activities,[37] and this activity has been confirmed using recombinant neuregulins on both rodent and human Schwann cells.[5,17] With the availability of recombinant neuregulins it has been possible to demonstrate additional activities on Schwann cells and their progenitors. Schwann cells are derived from the neural crest, and neuregulin (rhGGF2) has been shown to restrict multipotential neural crest stem cells to a glial cell fate.[26] Further, neuregulins promote the survival of both embryonic precursors and neonatal Schwann cells *in vitro*[20,22,25] and *in vivo* following nerve injury.[21,24] More recently, rhGGF2 has been shown to stimulate

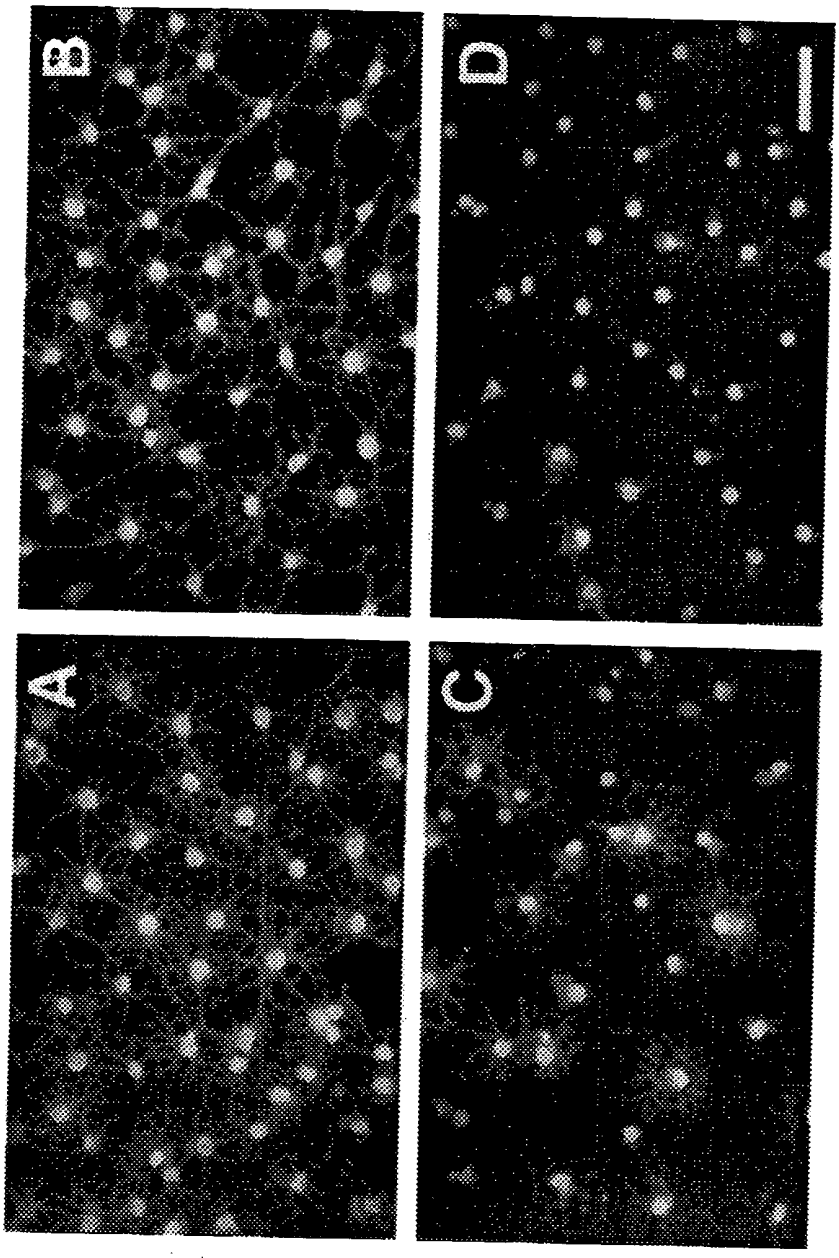
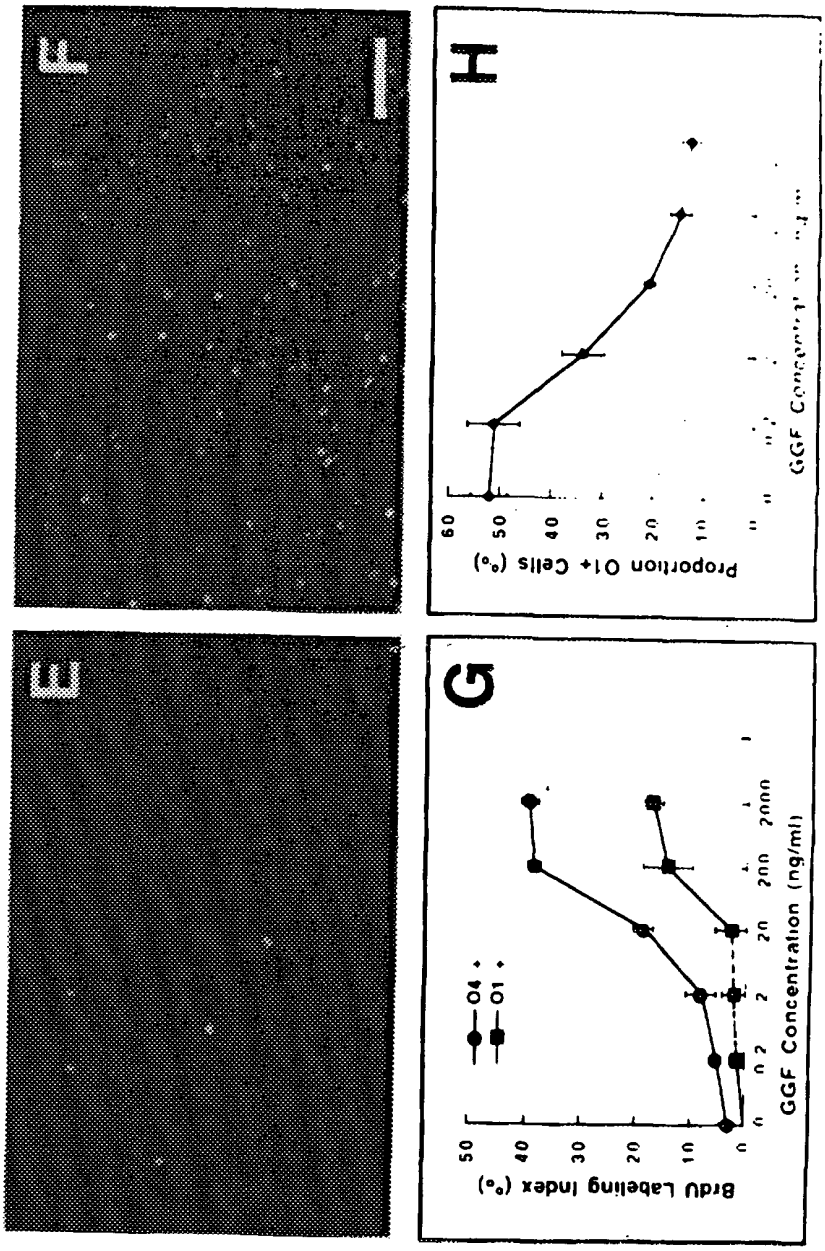


FIGURE 3. (*Continued on next page*).


F
I
H
Proportion O1+ Cells (%)
60
50
40
30
20
10
E
G
BrdU Labeling Index (%)
50
40
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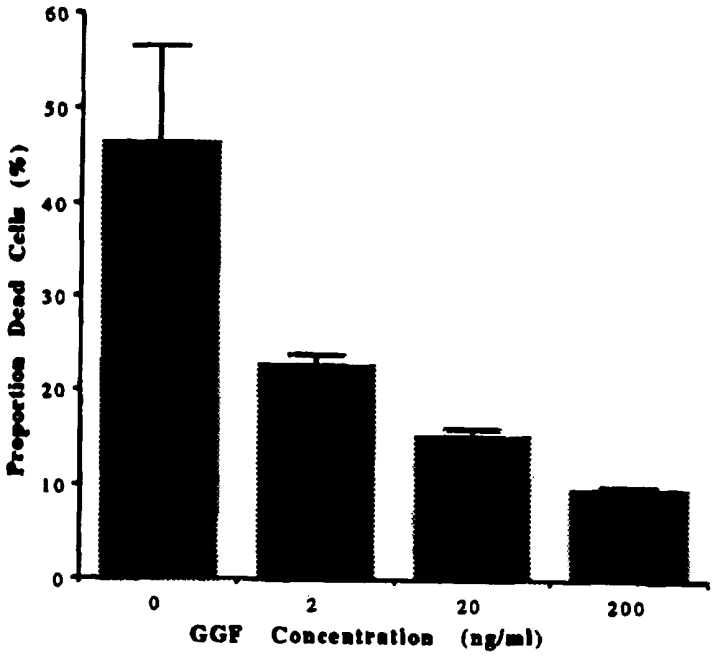
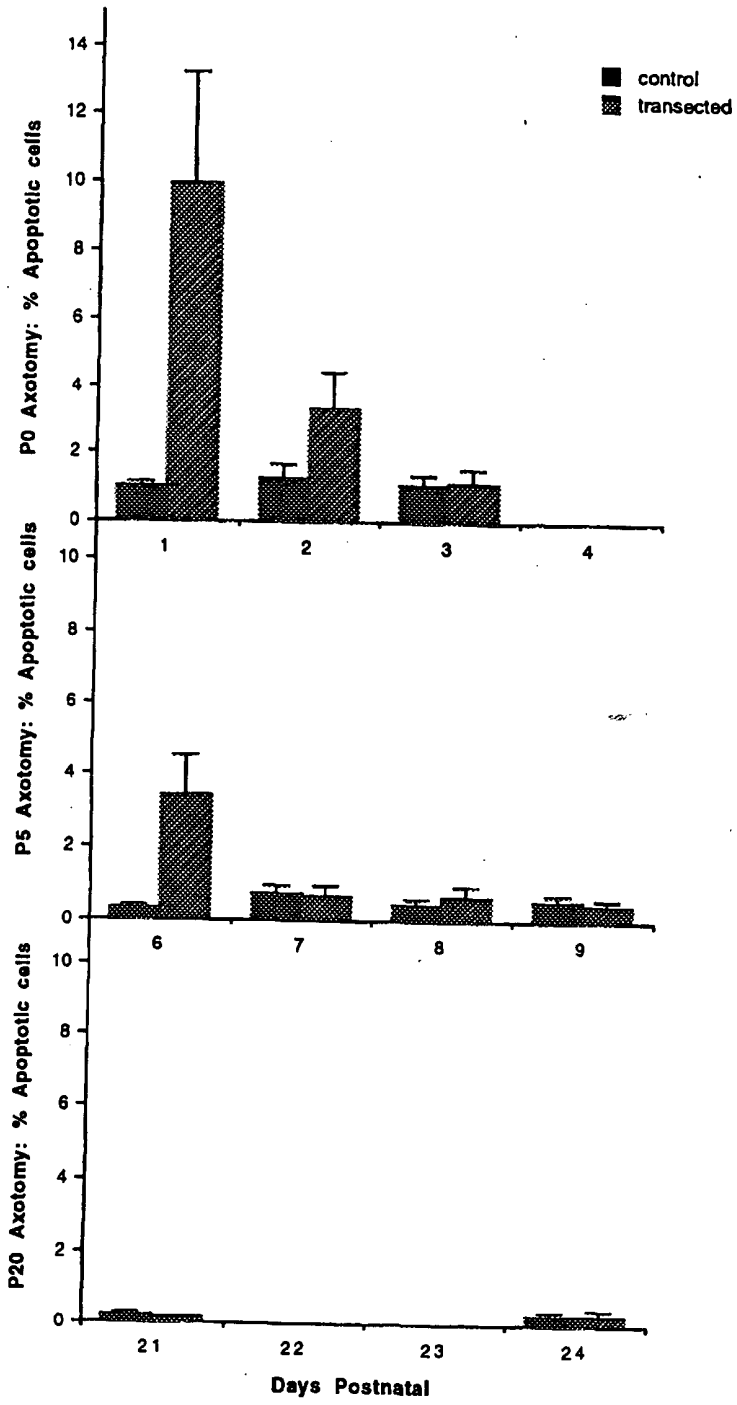


FIGURE 4. rhGGF2 promotes the survival of oligodendrocyte progenitors and maintains cell survival after serum withdrawal. O4+ cells (3 days in DM+) were switched to Dulbecco's modified Eagle's medium (DMEM) without any serum for 12 hours with increasing doses of rhGGF2. Dead cells were identified with a red nuclear stain; all other nuclei were stained blue. *Each bar* represents the mean ± SEM of duplicate wells from 3 independent experiments. (From Canoll *et al.*[23] Reprinted by permission from *Neuron.*)

FIGURE 3. Pleiotropic effects of rhGGF2 on cells in the oligodendrocyte lineage. **(A–F)** Cells were allowed to differentiate in DM+ for 3 days; cultures were then fed with DM+ without (*panels* A, C, E) or with 200 ng/ml of rhGGF2 (*panels* B, D, F) for 16 hours. In each case, BrdU was added during the last four hours. Cultures were fixed and stained for O4 (*panels* A, B), O1 (*panels* C, D) or BrdU (*panels* E, F) and counterstained with Hoechst dye to identify all cells in the field (*panels* A–D). Separate fields from a single experiment that are representative of the staining pattern of each antibody are shown. *Bar*, 50 μm (*panels* A–D); 100 μm (*panels* E, F). **(G)** O4+/O1− progenitors and O1+ oligodendrocytes exhibit different mitogenic responses to rhGGF2. The labeling index was determined for O4+ and O1+ cells after adding different concentrations of rhGGF2 for 16 hr; BrdU was added for the last 4 hr. Each point represents the mean ± SEM from 2 independent experiments. **(H)** Loss of O1 expression with increasing concentrations of rhGGF2. The percentage of O1+ cells was determined from cultures that were treated with increasing concentrations of rhGGF2 (described above in *panel* G). Each point represents the mean ± SEM from 2 independent experiments. (From Canoll *et al.*[23] Reprinted by permission from *Neuron.*)


control
transected
P0 Axotomy: % Apoptotic cells
P5 Axotomy: % Apoptotic cells
P20 Axotomy: % Apoptotic cells
0
2
4
6
8
10
12
14
1
2
3
4
6
7
8
9
21
22
23
24
Days Postnatal

Schwann cell migration in culture.[28] Here we describe axonal interactions that regulate Schwann cell death in developing peripheral nerve and further implicate neuregulin receptors and neuregulin in regulating Schwann cell number.[24]

Axotomy Greatly Increases Schwann Cell Apoptosis but Only during Early Development

We determined that apoptotic Schwann cells are present in sciatic nerve during the first two weeks of postnatal development, but not at later stages.[24] To determine whether axon-Schwann cell interactions regulate apoptosis, we transected the sciatic nerve to cause axonal degeneration. This was done both during postnatal days 0 and 5 (P0 and P5) and after P20 and P90, the normal period of apoptosis. In the P0, P5, and P20 animals, the distal nerve-stump was analyzed at 1, 2, 3, or 4 days after axotomy; in the P90 animals, this analysis was extended to include 8, 12, 24, and 58 days postlesion. Nonlesioned nerves from age-matched animals were used as controls. The sections were labeled for terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling (TUNEL) and counterstained with 4,6-diamidino-2-phenylidole (DAPI), so that the proportion of apoptotic nuclei could be determined.

The results of this experiment (FIG. 5) demonstrated that between 1 and 1.2% of the Schwann cells died by apoptosis during the first 3 days of development. For axotomy at P0, the number of apoptotic nuclei increased 10-fold 1 day postaxotomy, and then fell to within range of the controls by 3 days postaxotomy. Axotomy at P5 resulted in a smaller (3-fold) increase in the number of apoptotic nuclei at 1 day postaxotomy, followed by a decline to control levels. Axotomy at P20 (examined at 1 and 4 days postaxotomy) or P90 (examined at 1, 4, 8, 12, 24, and 60 days postaxotomy; data not shown) did not cause an increased number of apoptotic nuclei. These data indicate that there may be a window of susceptibility to axotomy-induced apoptosis in developing nerve, and the timing of this window corresponds to the normal period of Schwann cell apoptosis during development.

Apoptotic Schwann Cells Display a Predominately Premyelinating Phenotype

The first few days of postnatal development in the rat sciatic nerve are characterized by a commitment of some Schwann cells to initiate a program of myelin synthesis, while other cells are premyelinating. Thus we determined the phenotype of apoptotic cells using immunofluorescence to double-label TUNEL-positive nuclei with a panel of Schwann cell markers (data not shown). We stained sections of P0 and P6 nerves for the pan-Schwann cell marker S100β, the low-affinity nerve growth factor receptor (NGFR)/p75, which stains premyelinating and nonmyelinating

←

FIGURE 5. Axotomy increases Schwann cell apoptosis during early development. The distal nerve-stumps of sciatic nerves that were transected at P0, P5, and P20 (*crosshatched bars*), and age-matched, nonlesioned nerves (*solid bars*) were removed and processed for detection of apoptosis by the TUNEL assay. The data represents the mean percentage of apoptotic cells in 10 representative fields from 3–4 sections of each nerve. The *error bars* represent the standard error. (From Grinspan *et al.*[24] Reprinted by permission from the *Journal of Neuroscience.*)

Schwann cells, and periaxin, which is one of the earliest markers of myelinating Schwann cells.[38-40] Nearly all the apoptotic nuclei were found in S100β-positive cells, demonstrating that they were Schwann cells. Most TUNEL-positive nuclei were contained within the NGFR/p75-positive cells, indicating that they were in premyelinating Schwann cells.

We also determined the phenotype of TUNEL-positive cells in lesioned neonatal nerves. For these experiments, we analyzed nerves from P1 animals 1 day post-axotomy, at the peak of Schwann cell apoptosis, as the large number of TUNEL-positive cells permitted a quantitative analysis. We found that over 90% of the cells containing apoptotic nuclei were NGFR-positive, but less than 8% were periaxin-positive. Thus, in both normal and axotomized nerves, the overwhelming majority of apoptotic Schwann cells have a premyelinating phenotype, but only a subset of Schwann cells with this phenotype appear to undergo apoptosis.

Apoptosis following Axotomy and during Normal Development is Prevented by Administration of Exogenous Neuregulin

The observations that Schwann cell precursors die *in vitro* after the withdrawal of growth factors, including neuregulins,[20] and that neuregulins are made by peripheral nervous system (PNS) neurons and are axonally transported,[5,14] raise the possibility that neuregulin regulates Schwann cell apoptosis in developing nerves. Axotomy could cause apoptosis by removing the axonal source of neuregulin, and exogenous neuregulin could rescue axotomized Schwann cells, as recently observed at the neuromuscular junction.[21] We tested this idea by injecting neuregulin into the axotomized (left) leg so as to maximize the availability of neuregulin to the axotomized Schwann cells in the distal nerve-stump as follows. One group of newborn rat pups was axotomized and treated with neuregulin; one group was axotomized and treated with vehicle alone; one group was not axotomized and treated with neuregulin; one group was not axotomized and was injected with vehicle. All of the animals were sacrificed 24 hours after axotomy, the time at which the number of TUNEL-positive nuclei was maximal (FIG. 5). FIGURE 6 shows a representative field from an axotomized animal that received vehicle alone, and another field from an axotomized animal that was treated with neuregulin. Axotomized nerves that had received vehicle alone had many apoptotic Schwann cells, whereas axotomized nerves that received neuregulin had even fewer apoptotic cells than nonlesioned nerves.

These results are tabulated in TABLE 1. The most striking result was that in neuregulin-treated animals, axotomy did not increase the number of TUNEL-positive nuclei. This effect cannot be attributed to the vehicle, as there was 10-fold increase in the number of apoptotic Schwann cells following axotomy in animals treated with vehicle alone, comparable to the increase described above (FIG. 5) following axotomy at P0. Neuregulin also appeared to lower the endogenous rate of apoptosis in developing Schwann cells. In nonlesioned nerves, there were significantly fewer apoptotic Schwann cells following neuregulin administration than following treatment with vehicle alone ($p < 0.05$). In addition, the number of apoptotic Schwann cells found in the right sciatic nerve of the animal, which was never injected, tended to be lower in neuregulin-injected animals than in vehicle-injected animals, regardless of whether the left nerve was transected, but these differences did not achieve statistical significance. These results demonstrate that apoptotic Schwann cells can be rescued by neuregulin, and are thus not destined to die. Rather, the number of Schwann cells in developing nerves appears to be molded

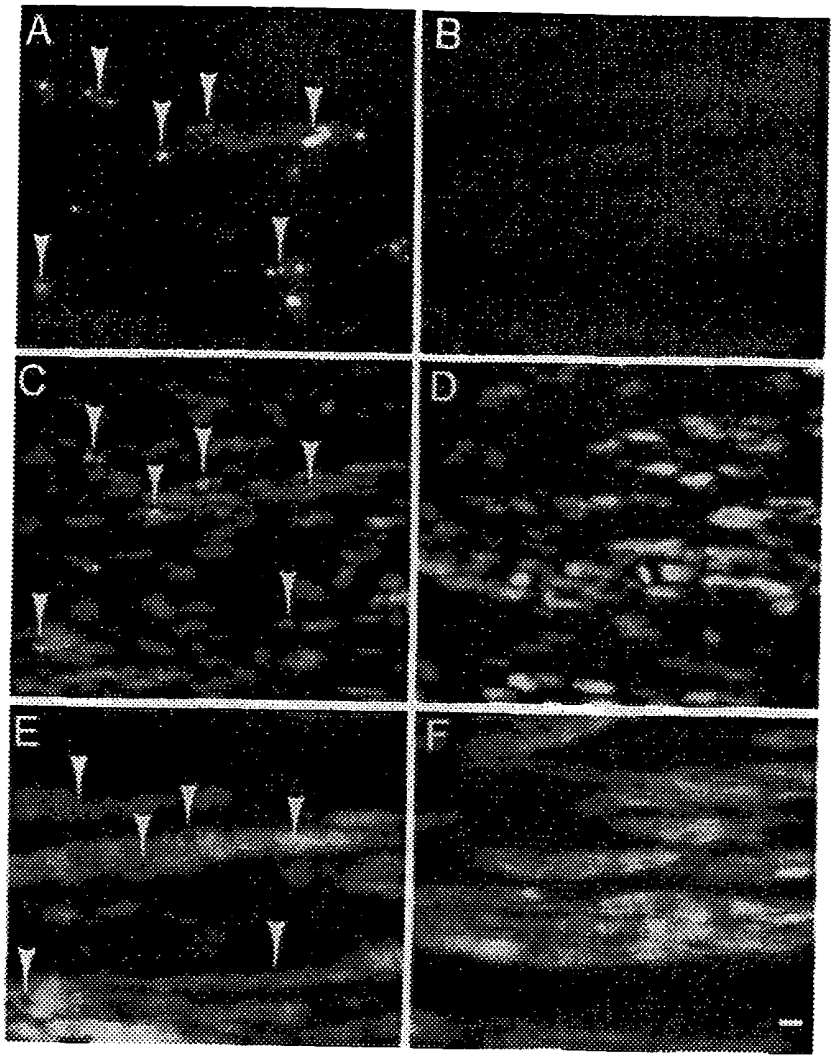


FIGURE 6. Neuregulin prevents Schwann cell apoptosis after axotomy. These are photomicrographs of longitudinal sections taken from sciatic nerve 1 day after transection. The *left panels* **(A, C, E)** are from a vehicle-only-treated animal, whereas the *right panels* **(B, D, F)** are from an animal treated with neuregulin. *Panels* (A) and (B) show TUNEL staining; TUNEL-positive nuclei are indicated by *arrowheads*. Note that there are no apoptotic nuclei in the neuregulin-treated nerve. *Panels* (C) and (D) show the DAPI counterstained nuclei; the *arrows* show that the TUNEL-positive nuclei (as in *panel* A) are fragmented. *Panels* E and F show S100b staining; the *arrowheads* indicate the location of the apoptotic nuclei, which appear as dark void within the Schwann cell cytoplasm. *Scale bar*: 10 mm. (From Grinspan *et al.*[24] Reprinted by permission from the *Journal of Neuroscience*.)

TABLE 1. Neuregulin Prevents Apoptosis in P1 Sciatic Nerves Axotomized for 1 Day[a]

Treatment	% TUNEL-Positive Nuclei	
	Transected (Left) Side	Nontransected (Right) Side
Transection, no neuregulin	9.44% ± 3.15	1.66% ± 0.28
Transection, plus neuregulin	0.72% ± 0.24*	0.97% ± 0.23**
No transection, no neuregulin	1.68% ± 0.54	2.40% ± 0.41
No transection, plus neuregulin	0.38% ± 0.23[#]	1.68% ± 0.39[##]

[a] Newborn rats were divided into 4 treatment groups, 3 animals per group (see Methods). One day after transection, the distal stumps from transected nerves, the corresponding segment from nonlesioned nerves, and the contralateral nerves, were removed and processed for the TUNEL assay. TUNEL-positive nuclei were counted, and their percentage of the total was calculated. The % TUNEL-positive nuclei in the left and right sciatic nerves from animals that received neuregulin was compared to that in animals that received vehicle alone by a paired, two-tailed *t* test. (From Grinspan *et al.*[24] Reprinted by permission from the *Journal of Neuroscience.*)

* The number of apoptotic cells in transected nerves from animals that received neuregulin was significantly less than that in transected nerves from animals that received vehicle alone ($p < 0.0001$).

[#] The number of apoptotic cells in nontransected nerves from animals that received neuregulin was significantly less than that in nontransected nerves from animals that received vehicle alone ($p < 0.05$).

**,[##] Although neuregulin reduced the percentage of TUNEL-positive cells in the contralateral leg in both the transected and the nontransected animals, this reduction did not achieve statistical significance in either group.

by axon-Schwann cell interactions. Neuregulin may thus be one of the axonally-derived factors which mediate Schwann cell survival.

Neuregulin Promotes Expression of Neurotrophic Activity by Schwann Cells

Data from *in vitro* studies have shown that rhGGF2 elicits the production of soluble neurotrophic activity from Schwann cells that support peripheral neuron survival and outgrowth.[28] In those studies a "tube culture" system was developed in which a portion of a peripheral ganglion (superior cervical ganglion, SCG) was placed at one end of a polyethylene tube filled with medium containing various concentrations of rhGGF2 in a three-dimensional type I collagen matrix. Neurite outgrowth was markedly increased in the presence of rhGGF2 in the collagen tube culture system. However, when analyzed in cultures of pure SCG neurons grown in a traditional two-dimensional format, rhGGF2 neither promoted survival nor shifted NGF dose responsiveness; there was no obvious effect of rhGGF2 on neurite outgrowth.[28] Thus it is unlikely that the effects of rhGGF2 on neurite outgrowth are direct. To determine if neuregulin might elicit the expression of neurotrophic factors that might account for the neurite outgrowth observed in the experiments described above, we assayed media conditioned by Schwann cells treated with rhGGF2. Indeed, as shown in FIGURE 7, rhGGF2 induced the production of neurotrophic activity by Schwann cells, which stimulated the survival of isolated SCG neurons. This induced activity increased with dose of rhGGF2. Because of the absence of an observable effect of rhGGF2 on neurite outgrowth from purified SCG neurons, we suggest that the rhGGF2-induced outgrowth observed in the tube cultures derives from neurotrophic activity induced in Schwann cells present in the explant.[28]

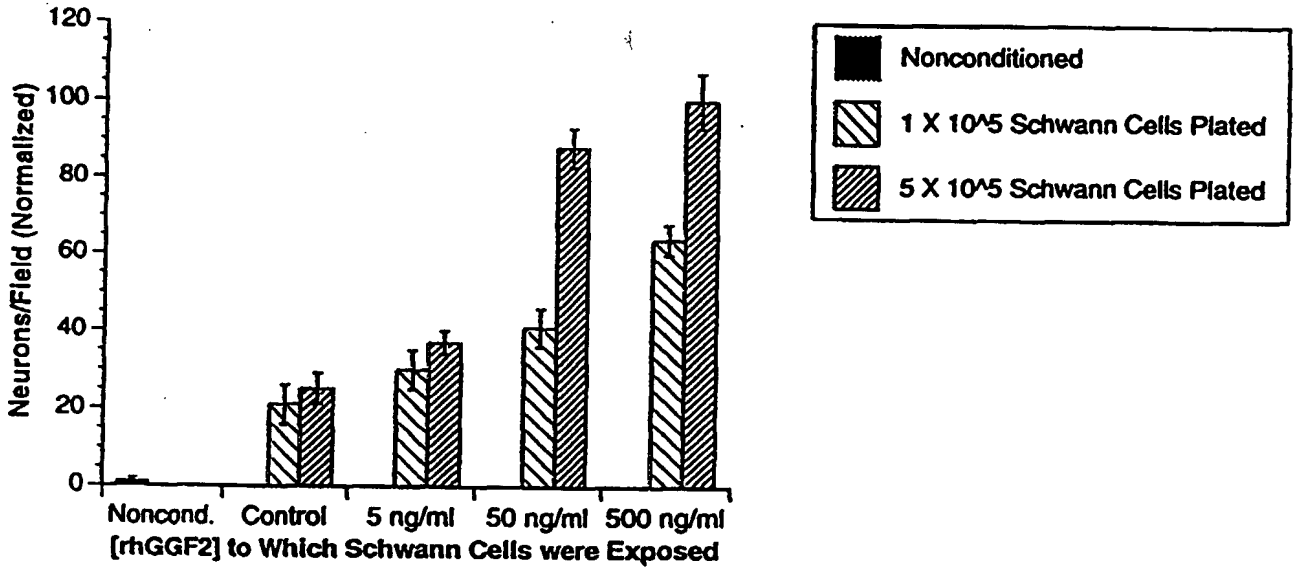


FIGURE 7. Neuregulin promotes expression of soluble neurotrophic activity by Schwann cells. Schwann cells (initial plating densities: 1×10^5 cells, *shaded bars*; 5×10^5 cells, *crosshatched bars*) were cultured in rhGGF2 at various concentrations, and the conditioned media were collected after 5 days. After dilution of the conditioned media by a factor proportional to the final Schwann cell numbers (thus normalizing for constitutive secretions), the media were used to culture SCG neurons in a 2-day survival assay (mean ± SEM, n = 10). (Adapted from Mahanthappa *et al.*[28])

DISCUSSION

Neuregulins as Indirect Neurotrophins

Reciprocal cell-cell interactions are involved in various aspects of neural development and function. We present several lines of evidence that neuronally-expressed neuregulins regulate glial cell number, which in turn provide trophic support for neurons. Thus neuregulins may act as indirect neurotrophic factors, and in this way have potential as neuroprotective agents. For example, the expression of neurotrophin-3 (NT3) was upregulated 40-fold in glial cells of embryonic sympathetic ganglia treated with rhGGF2 and blocking antibodies to rhGGF2 diminished the neuronal signaling that induced NT3 expression.[37] Further, conditioned media from Schwann cells treated with rhGGF2 may contain other soluble neurotrophic activities that promoted survival of sympathetic neurons (see FIG. 7). Since NGF is a survival factor for SCG neurons, and Schwann cells express this neurotrophin, NGF is a candidate for rhGGF2-induced neurotrophic activity.

Neurons are dependent for their survival on neurotrophic factors provided by targets and by support cells. The distinct patterns of neurotrophin dependence are dictated largely by the expression of trk receptors. In general, neurons that express trkA are supported by NGF; trkB-expressing neurons are dependent on brain-derived neurotrophic factor (BDNF) or NT4/5, and; trkC-expressing neurons are supported by NT3 (for review, see Ref. 41). Because of the specificity of these survival requirements, no single neurotrophin can be expected to directly rescue all of the various peripheral neurons that are afflicted either in chronic neurological diseases or acute injury.

Schwann cells represent a major source of trophic support for peripheral neurons. Schwann cells are known to express all of the neurotrophins as well as insulin-like growth factor (IGF), ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), and glial cell line-derived neurotrophic factor (GDNF), and produce increased quantities of most factors after injury.[42,43,43b] By increasing Schwann cell number and/or by inducing the expression of multiple growth factors produced by Schwann cells, an array of neurotrophic activities can be elicited to support all of the various peripheral fiber types.

Neuregulins as Potential Drugs for Neurological Disorders

The cardinal feature of many neurological disorders including amyotrophic lateral sclerosis, Alzheimer's disease, and Parkinson's disease is neuronal loss. By promoting neuronal survival growth factors might be expected to slow the progression and possibly even reverse the course of these diseases. In animal models of motor neuron injury by axotomy BDNF, CNTF and GDNF individually rescue motor neurons from death. These factors can also sustain a variety of CNS neurons. Both NGF and NT3 have been evaluated in animal models of peripheral neuropathy with positive results.[48] As a result of the encouraging results obtained in preclinical studies, several of these growth factors now have advanced into clinical trials. IGF-I has been tested in a wide range of models and may be approved as a treatment for motor neuron disease.[49] Since neuregulins have pleiotrophic actions in the nervous system, they also have potential in the treatment of a variety of neurological disorders.

Owing to their effects on glial cells and skeletal muscle, neuregulins may be useful in the treatment of peripheral neuropathy, multiple sclerosis and myopathy

(*e.g.*, see Ref. 2). Given the spectrum of biological activities attributed either directly or indirectly to neuregulins *in vitro* and *in vivo*, furthermore, it seems that these growth factors might fit into a treatment regimen for acute neurological conditions, such as stroke or traumatic brain and spinal cord injury. In this setting neuregulins might be used in combination therapy with neuroprotective ion channel blockers, such as CERESTAT®, and agents applied to restore cerebral blood flow, such as tissue plasminogen activator (TPA). Testing of neuregulins in the appropriate animal models of focal ischemia and spinal cord injury would seem to be the next logical step in this direction. Continued evaluation of this growth factor in additional models of neurological disorders accompanied by further progress in understanding the basic neurobiology of neuregulins and their receptors will help to advance rhGGF2 toward eventual clinical trials.

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